

CLAIMS

What is claimed is:

1. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
 - 5 a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is
 - 10 selected from the group consisting of *narGHJI*, *csn*, *yncM*, *yvyD*, *yvaWXY*, *ydjL*, *sunA*, and *yolIJK* and homologues thereof; and
 - 15 b) growing the transformed *Bacillus sp* cell of step (a) in the absence of oxygen wherein the chimeric gene of step (a) is expressed.
2. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
 - 20 a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is
 - 25 selected from the group consisting of *narGHJI*, *csn*, *yncM*, *yvyD*, *yvaWXY*, *ydjL*, *sunA*, and *yolIJK* and homologues thereof;
 - 25 b) growing the transformed *Bacillus sp.* cell of step (a) in the presence of oxygen whereby the cell density is increased; and
 - 30 c) removing oxygen from the transformed *Bacillus sp.* cell or step (b) whereby the chimeric gene is expressed.
3. A method according to Claim 2 wherein after step (c) oxygen is re-supplied to the transformed *Bacillus sp.* cell.
4. A method according to either of Claims 1 or 2 wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of SEQ ID NOs:1-15.
5. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
 - 35 a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of

- interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of *feuABC*, *ykuNOP*, and *dhbABC*, and homologues thereof; and
- 5 b) growing the transformed *Bacillus sp* cell of step (a) in the absence of oxygen and in the presence of nitrite wherein the chimeric gene of step (a) is expressed.
6. A method according to Claim 5 wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of SEQ ID NOs:16-24.
- 10 7. A method according to Claim 6 wherein the concentration of nitrite is from about 1mM to about 10 mM.
8. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
- 15 a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of *ycgMN*, *dhaS rapF*, *rapG*, *rapH*, *rapK*, *yqhIJ*, *yveKLMNOPQST*, *yhfrSTUV*, *csn*, *yncM*, *yvyD*, *yvaWXY*, *ydjL*, *sunA*, and *yolIJK*, and homologues thereof; and
- 20 b) growing the transformed *Bacillus sp* cell of step (a) in the presence of oxygen until the cell reaches about T0 of the stationary phase wherein the chimeric gene of step (a) is expressed.
- 25 9. A method according to Claim 8 wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of SEQ ID NOs:75, 76, 25-49, and 5-15.
- 30 10. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
- 35 a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is

selected from the group consisting of *acoABCL*, and *glvAC*, and homologues thereof; and

- 5 b) growing the transformed *Bacillus sp* cell of step (a) in the presence of oxygen until the cell reaches about T1 of the stationary phase wherein the chimeric gene of step (a) is expressed.

11. A method according to Claim 10 wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of SEQ ID NOs:41-44 and 50-51.

- 10 12. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:

- 15 a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of *yxjCDEF*, *ynGEFGHI*, *yjmCDEFG*, *ykfABCD*, and *yodOPRST*; and homologues thereof; and
- 20 b) growing the transformed *Bacillus sp* cell of step (a) in the presence of oxygen until the cell reaches about T3 of the stationary phase wherein the chimeric gene of step (a) is expressed.

25 13. A method according to Claim 12 the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of SEQ ID NOs:52-74.

14. A method according to any of Claims 1, 2 or 3 wherein the expression of the chimeric gene is down-regulated at T0 of the stationary phase.

30 15. A method according to any one of Claims 1, 2, 3, 4, 8, 10 and 12 wherein the *Bacillus sp.* cell is selected from the species consisting of *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus brevis*, *Bacillus megaterium*, *Bacillus intermedius*, *Bacillus thermoamyloliquefaciens*, *Bacillus amyloliquefaciens*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus sphaericus*, *Bacillus*

35 *stearothermophilus*, *Bacillus laterosporus*, *Bacillus acidocaldarius*, *Bacillus pumilus*, and *Bacillus pseudofirmus*.

16. The method according to any one of Claims 1, 2, 3, 4, 8, 10 and 12, wherein the coding region of interest is selected from the group consisting of *crtE*

crtB, *pds*, *crtD*, *crtL*, *crtZ*, *crtX crtO*, *phaC*, *phaE*, *efe*, *pdC*, *adh*, genes encoding limonene synthase, pinene synthase, bornyl synthase, phellandrene synthase, cineole synthase, sabinene synthase, and taxadiene synthase.

5 17. A method for monitoring the state of the cell metabolism of a *Bacillus* *sp.* culture comprising:

- a) providing a culture of actively growing *Bacillus* *sp.* cells; and
- b) measuring the expression levels of a pool of genes isolated from the *Bacillus* cells of step (a), the pool of genes comprising
10 *narGHJI*, *feuABC*, *ykuNOP*, *dhbABC*, *ydjL*, *sunA*, *yolIJK*, *csn*, *yncM*, *yvyD*, *yvaWXY*, *yhfRSTUV*, *yveKLMNOPQST*, *dhaS*,
rapF, *rapG*, *rapH*, *rapK*, *ycgMN*, *yqhIJ*, *glvAC*, *acoABCL*,
15 *yxjCDEF*, *yngEFGHI yjmCDEFG*, *ykfABCD*, *yodOPRST*, *alsT*,
and *yxeKLMN*, and homologues thereof.

15 18. A method according to Claim 17 wherein a pool of genes isolated from the *Bacillus* cells is selected from the group consisting of SEQ ID NOs:1-81.

19. A method according to Claim 17 wherein the measuring of gene expression levels is accomplished using a format selected from the group consisting of northern blots, nuclease protection assay or primer extension assays.

20 20. A method according to Claim 19 wherein the measuring of gene expression levels is accomplished using a nucleic acid microarray having the genes *narGHJI*, *feuABC*, *ykuNOP*, *dhbABC*, *ydjL*, *sunA*, *yolIJK*, *csn*, *yncM*, *yvyD*,
yvaWXY, *yhfRSTUV*, *yveKLMNOPQST*, *dhaS*, *rapF*, *rapG*, *rapH*, *rapK*, *yqhIJ*,
glvAC, *acoABCL*, *yxjCDEF*, *yngEFGHI yjmCDEFG*, *ykfABCD*, *yodOPRST*, *alsT*,
25 and *yxeKLMN*, and homologues thereof, contained therein.

25 21. A method according to Claim 17 wherein the *Bacillus* *sp.* cell is selected from the species consisting of *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus brevis*, *Bacillus megaterium*, *Bacillus intermedius*, *Bacillus thermoamyloliquefaciens*, *Bacillus amyloliquefaciens*,
Bacillus circulans, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus*
30 *sphaericus*, *Bacillus stearothermophilus*, *Bacillus laterosporus*, *Bacillus acidocaldarius*, *Bacillus pumilus*, and *Bacillus pseudofirmus*.

22. A method according to Claim 17 wherein the actively growing culture is grown in the absence of oxygen and the expression of genes *narGHJI*, *ydjL*, *sunA*, *yolIJK*, *csn*, *yncM*, *yvyD*, and *yvaWXY* are up-regulated in the log phase.

35 23. A method according to Claim 17 wherein the actively growing culture is grown in the absence of oxygen and in the presence of nitrite and the expression of genes *feuABC*, *ykuNOP*, and *dhbABC* are up-regulated in the log phase.

24. A method according to either of Claims 22 or 23 wherein the expression of genes *narGHJI* is down-regulated at about T0 of the stationary phase.

25. A method according to Claim 17 wherein the actively growing culture is grown in the presence of oxygen and the expression of genes *ycgMN*, *yqhIJ*, *ydjL*, *sunA*, *yolIJK*, *csn*, *yncM*, *yvyD*, *yvaWXY*, *yhfRSTUV*, *yveKLMNOPQST*, *dhaS*, *rapF*, *rapG*, *rapH*, *rapK*, are up-regulated at about T0 of the stationary phase.

26. A method according to Claim 17 wherein the actively growing culture is grown in the presence of oxygen and the expression of genes, *acoABCL* and *glvAC* are up-regulated at about T1 of the stationary phase.

27. A method according to Claim 17 wherein the actively growing culture is grown in the presence of oxygen and the expression of genes, *yxjCDEF*, *yngEFGHI*, *yjmCDEFG*, *ykfABCD*, and *yodOPRST* are up-regulated at about T3 of the stationary phase.

28. A method according to Claim 17 wherein the actively growing culture is grown in the presence of oxygen and the expression of genes, *alsT* and *yxkLMN* are down-regulated at stationary phase or under nutrient-limiting conditions.